REMARKS

Claims 69-70 are pending in the present application and at issue. Claim 70 has been amended to clarify the claimed invention. The scope of the claim has not changed.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested.

I. The Rejection of Claims 69-70 under 35 U.S.C. 112

Claims 69-70 are rejected under 35 U.S.C. 112, first paragraph, "because the specification, while being enabling for DNA encoding a Savinase or Savinase library enzyme, does not reasonably provide enablement for the broadly claimed method using a gene encoding a diversified library of protein variants." This rejection is respectfully traversed.

It is also well settled that "a specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first paragraph of section 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support." *In re Marzocchi*, 169 U.S.P.Q. 367, 369 (C.C.P.A. 1971).

Moreover, "[a]ny assertion by the Patent Office that the enabling disclosure is not commensurate in scope with the protection sought must be supported by evidence or reasoning substantiating the doubts so expressed." *In re Dinh-Nguyen*, 181 U.S.P.Q. 46, 47 (C.C.P.A. 1974). Thus, the burden is upon the Patent Office to set forth reasonable grounds in support of its contention that a claim reads on inoperable subject matter). See *In re Stark*, 172 U.S.P.Q. 402, 406 n. 4 (C.C.P.A. 1972).

It is also well settled that a patent need not teach and preferably omits what is well known in the art. *Spectra-Physics Inc. v. Coherent Inc.*, 3 U.S.P.Q.2d 1737 (Fed. Cir. 1987).

Applicants submit that the specification complies with the enablement rejection.

The claimed invention relates to methods for selecting a variant of a protein with reduced immunogenicity. Proteins and DNA sequences encoding same are well known in the art. Furthermore, at pages 30-38, the specification describes numerous proteins for use in the methods of the present invention. Moreover, it is routine for one of ordinary skill in the art to generate libraries of variants of a protein, e.g., as described at pages 40-44 of the specification.

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For the foregoing reasons, Applicants submit that th claims overcome this rejection under 35 U.S.C. 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

II. The Rejection of Claims 69-70 under 35 U.S.C. 112

Claims 69-70 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. Specifically, the Office states:

> The specification fails to provide an adequate written description of a method for selecting a variant protein with reduced immunogenecity by screening using competitive ELISA assay. In conjunction, there is no description in the specification as to a diverse DNA library of genes that encodes a variant protein. It does not describe how a diverse DNA library of genes is generated, the source of the diverse kinds of genes and/or identification of the genes for library formation.

This rejection is respectfully traversed.

As explained in Section I above, proteins and DNA sequences encoding same are well known in the art. Furthermore, at pages 30-38, the specification describes numerous proteins for use in the methods of the present invention. Moreover, it is routine for one of ordinary skill in the art to generate libraries of variants of a protein, e.g., as described at pages 40-44 of the specification.

Moreover, competitive ELISA is a well known assay. Applicants enclose a copy of an article from Current Protocols in Immunology, which describes competitive ELISA.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

III. The Rejection of Claims 69-70 under 35 U.S.C. 112

Claims 69-70 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite. This rejection is respectfully traversed in part.

First, the Office objected to the terms "reduced," "capacity," and "diversified" as being relative terms. This rejection is respectfully traversed.

Applicants submit that these terms are clear to one of ordinary skill in the art. For example, the specification describes that a protein variant has "reduced immunogenicity" relative to the protein. Furthermore, the specification discloses that "the antibody binding capacity" is measured using competitive ELISA. Finally, the term "diversified library" would be understood by one of ordinary skill in the art as having diff rent variants.

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Second, the Office objected to the term "the sampl s" for lacking antecedent basis. Claim 69 has been amended to provide antecedent basis for this term. Therefore, this rejection has been overcome.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

IV. The Rejection of Claims 69-70 under the Doctrine of Obviousness-Type Double Patenting

Claims 69-70 are rejected under the doctrine of obviousness-type double patenting as being unpatentable over claims 55-73 of Application No. 09/417,608 and over claims 1-7, 11-14, 20-21 and 40 of Application No. 09/694,173. This rejection is respectfully traversed.

Claim 55 of the 608 application reads as follows:

> 55. A method of producing a protein variant, comprising the steps of:
> (a) subjecting a random peptide display package library to one or more antibodies to identify peptides that bind to the one or more of the antibodies;
> (b) identifying one or more epitope patterns by aligning the peptides that bind to the one or more of the antibodies with each other;
> (c) obtaining a three-dimensional structure of a parent protein;
> (d) identifying the one or more epitope patterns on the three-dimensional structure of the parent protein;
> (e) identifying an epitope area of amino acids situated within 5 Å of any amino acid of the one or more epitope patterns of the parent protein; and
> (f) modifying one or more amino acids identified in step (e) of the parent protein to form the protein variant, wherein the protein variant retains functionality of the parent protein and has a lower immunogenicity than the parent protein.

Claim 1 of the 173 application reads as follows:

> 1. A method for high throughput screening (HTS) of a population of host cells for production of a molecule of interest, the method comprising the steps of:
> (a) arranging the host cells in a spatial array so each position in the spatial array is occupied by one cell,
> (b) cultivating the host cells in an HTS process, wherein the host cells are cultivated under growth conditions that ensure minimal variation in the concentration or amount of the molecule of interest between all positions in the spatial array,
> (c) assaying each array position for production of the molecule of interest, and
> (d) selecting the cells from those positions where the mol cule of interest was produced, as determined in step c).

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The claims of the 608 and 173 applications do not use competitive ELISA, as recited in the claims of the present invention. Thus, the inventions claimed herein are patentably distinct from the 608 and 173 applications.

V. The Rejection of Claims 69-70 under 35 U.S.C. 102

Claims 69-70 are rejected under 35 U.S.C. 102(b) as being anticipated by Jespers et al. (J. Mol. Biol., 269: 704-718 (1997)). This rejection is respectfully traversed.

Jespers et al. disclose a method of epitope mapping, comprising preparing a randomized library of staphylokinase mutants by error-prone PCR, phage display, and negative selection on binding to antibodies. The staphylokinase mutants were analyzed by phage ELISA.

However, Jespers et al. do not disclose a method comprising the use of competitive ELISA to identify variants with reduced immunogenicity. Applicants therefore submit that this rejection has been overcome.

VI. The Rejection of Claims 69-70 under 35 U.S.C. 103

Claims 1-20 are rejected under 35 U.S.C. 103 as being unpatentable over Jespers et al. or Williams et al. (J. Immunological Methods, 213: 1-17 (1998)). This rejection is respectfully traversed.

Jespers et al. is discussed in Section V above.

Williams et al. disclose a method for identifying linear epitopes of beta lactoglobulin (BLG) using PEPSCAN and phage display. Specifically, Williams et al. raise an antibody against BLG and subject a library of randomized short peptides to the antibody. Williams et al. then align BLG and the nanopeptides that bind to the antibody. Williams et al. use phage ELISA to screen clones for binding to anti-BLG IgG.

However, Williams et al. also do not disclose or teach a method comprising the use of competitive ELISA to identify variants with reduced immunogenicity.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 103. Applicants respectfully request reconsideration and withdrawal of the rejection.

VII. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to

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contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

Date: November 19, 2003

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13. Wash plates as in steps 9 to 11.

After final rinsing, plates may be wrapped in plastic wrap and stored for months at 4°C prior to adding substrate.

Add substrate and measure hydrolysis

14. Add 75 µl MUP or NPP substrate solution to each well and incubate 1 hr at room temperature.

15. Monitor hydrolysis qualitatively by visual inspection or quantitatively with a microtiter plate reader (see below). Hydrolysis can be stopped by adding 25 µl of 0.5 M NaOH.

    a. Visually, hydrolysis of NPP can be detected by the appearance of a yellow color. If using a microtiter plate reader to measure NPP hydrolysis, use a 405-nm filter.

    b. Visually, hydrolysis of MUP can be monitored in a darkened room by illumination with a long-wavelength UV lamp. If using a microtiter plate spectrofluorometer to measure MUP hydrolysis, use a 365-nm excitation filter and a 450-nm emission filter.

The fluorogenic system using the MUP substrate is 10 to 100 times faster than the chromogenic system using NPP. Furthermore, the rate of spontaneous hydrolysis of MUP is much lower than that of NPP.

To detect bound antibodies that are present at low concentration, measure hydrolysis at a later time. To calculate when to measure hydrolysis the second time, remember that the amount of hydrolysis is almost directly proportional to the time of hydrolysis. For example, if the hydrolysis in the wells of interest reads 200 at 1 hr and a reading of 2000 is desired, incubate the plate ~10 hr before taking the second reading.

ALTERNATE PROTOCOL

DIRECT COMPETITIVE ELISA TO DETECT SOLUBLE ANTIGENS

This assay is used to detect or quantitate soluble antigens and is most useful when both a specific antibody and milligram quantities of purified or semi-purified antigen are available (Fig. 2.1.2). To detect soluble antigens, plates are coated with antigen and the binding of specific antibody-enzyme conjugates to antigen-coated plates is inhibited by test solutions containing soluble antigen. After incubation with mixtures of the conjugate and inhibitor in antigen-coated wells, unbound conjugate is washed away and substrate is added. The amount of antigen in the test solutions is proportional to the inhibition of substrate hydrolysis and can be quantitated by interpolation onto an inhibition curve generated with serial dilutions of a standard antigen solution.

The direct assay may also be adapted as an indirect assay by substituting specific antibody for specific antibody-enzyme conjugate. The amount of specific antibody bound is then detected using a species-specific or isotype-specific conjugate as a tertiary reactant.

Additional Materials

Specific antibody–alkaline phosphatase conjugate (second support protocol)
Standard antigen solution
Test antigen solutions
Round- or cone-bottom microtiter plates

1. Determine the optimal concentration of coating reagent and antibody–alkaline phosphatase conjugate by criss-cross serial-dilution analysis in which the concentrations of both the antigen (coating reagent) and the conjugate (developing reagent) are

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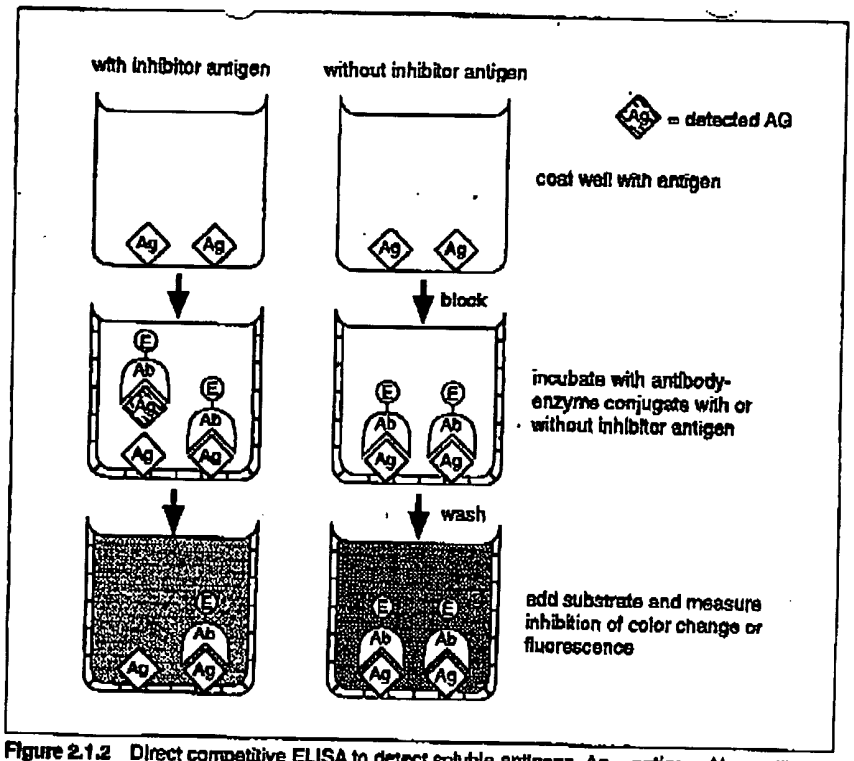

Figure 2.1.2 Direct competitive ELISA to detect soluble antigens. Ag = antigen; Ab = antibody; E = enzyme.

varied (see first support protocol). Prepare a 2× conjugate solution by diluting the specific antibody–alkaline phosphatase conjugate in blocking buffer to twice the optimal concentration.

The final concentration is usually 25 to 500 ng antibody/ml. Prepare 3 ml antibody–alkaline phosphatase conjugate for each plate.

2. Coat and block wells of an Immulon microtiter plate with 50 µl antigen solution as in steps 2 to 7 of the basic protocol.

3. Prepare six 1:3 serial dilutions of standard antigen solution in blocking buffer (see first support protocol for preparation of serial dilutions)—these antigen concentrations will be used in preparing a standard inhibition curve (see step 10).

Antigen concentrations should span the dynamic range of inhibition. The dynamic range of inhibition is defined as that range of inhibitor concentrations wherein changes of inhibitor concentration produce detectable changes in the amount of inhibition. The dynamic range of inhibition is empirically determined in an initial assay in which antigen concentration is typically varied from the micromolar (10^{-6} M) to the picomolar (10^{-12} M) range. For most protein antigens, initial concentration should be ~10 µg/ml, followed by nine 1:4 serial dilutions in blocking buffer. These antigen dilutions are assayed for their ability to inhibit the binding of conjugate to antigen-coated plates under standard assay conditions. From this initial assay, six 1:3 antigen dilutions spanning the dynamic range of inhibition are selected for further use as standard antigen-inhibitor dilutions. Prepare ≥75 µl of each dilution for each plate to be assayed.

Inhibitor curves are most sensitive in the region of the curve where small changes in inhibitor concentrations produce maximal changes in the amount of inhibition. This

region of the curve normally spans 15% to 85% inhibition. In most systems, this range of inhibition is produced by concentrations of inhibitor between 1 and 250 ng/mL.

4. Mix and incubate conjugate and inhibitor by adding 75 µl of 2× conjugate solution (from step 1) to each well of a round- or cone-bottom microtiter plate, followed by 75 µl inhibitor—either test antigen solution or standard antigen solution (from step 3). Mix the conjugate and inhibitor solutions by pipetting up and down in the pipet tip three times (see annotation to step 8 in the basic protocol) and incubate ≥30 min at room temperature.

   For accurate quantitation of the amount of antigen in the test solutions, test antigen solutions should inhibit conjugate binding between 15% to 85%. It may be necessary to assay two or three different dilutions of the test solutions to produce inhibitions within this range.

5. Prepare uninhibited control samples by mixing equal volumes of 2× conjugate solution and blocking buffer.

6. Transfer 50 µl of the mixture of conjugate plus inhibitor (from step 4) or conjugate plus blocking buffer (from step 5) to an antigen-coated plate (from step 2) and incubate 2 hr at room temperature.

   If samples are to be assayed in duplicate, the duplicates should be in adjacent columns on the same plate. Reserve column 11 for uninhibited control samples (step 5) and column 12 for substrate alone without any conjugate. If the concentration of antigen in the test samples is to be accurately quantitated, dilutions of homologous antigen solutions (step 3) should be included on each plate.

7. Wash plate as in steps 9 to 11 of the basic protocol.

8. Add 75 µl of MUP or NPP substrate solution to each well and incubate 1 hr at room temperature.

9. Read plates on the microtiter plate reader after ≥1 hr, at which time enough substrate has been hydrolyzed in the uninhibited reactions to permit accurate measurement of the inhibition.

10. Prepare a standard antigen-inhibition curve constructed from the inhibitions produced by the dilutions of the standard antigen solutions from step 3. Plot antigen concentration on the *x* axis, which is a log scale, and fluorescence or absorbance on the *y* axis, which is a linear scale.

11. Interpolate the concentration of antigen in the test solutions from the standard antigen-inhibition curve.

    The dynamic range of the inhibition curve may deviate from linearity if the specific antibodies are heterogeneous and possess significantly different affinities or if the standard antigen preparation contains heterogeneous forms of the antigen. Antigen concentration in test samples can be accurately interpolated from the inhibition curve as long as the test antigen is antigenically identical to the standard antigen and the concentration of test antigen falls within the dynamic range of inhibition.

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